PB81-184731

Acute Toxicity of Ammonia and Nitrite to Cutthroat Trout Fry

Montana State Univ., Bozeman

Prepared for

Environmental Research Lab.-Duluth
Monticello, MN

Apr 81

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1. REPORT NO.	2.	3. RECI
EPA-600/J-78-182	Journal Article	PB81-184731

4. TITLE AND SUBTITLE	5. REPORT DATE
Acute Toxicity of Ammonia and Nitrite to Cutthroat Trout Fry	April 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Thurston, R. V., R. C. Russo, and C. E. Smith	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Fisheries Bioassay Laboratory Montana State University Bozeman, Montana 59715	11. CONTRACT/GRANT NO. R800861
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth Monticello Ecological Research Station Monticello, Minnesota 55362	14. SPONSORING AGENCY CODE EPA-600/03

15. SUPPLEMENTARY NOTES
Trans. Am. Fish. Soc. 107(2): 361-368 (1978)

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

PRODUCT OF:
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	10
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA-600/J-78-182
Journal Article

Trans. Am. Fish. Soc., 107(2): 361–368, 1978

Acute Toxicity of Ammonia and Nitrite to Cutthroat Trout Fry

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ABSTRACT

The toxicity of ammonia and of nitrite was tested on cutthroat trout (*Salmo clarki*) fry (1–3 g) for periods up to a month in eight laboratory flow-through bioassays. Median lethal concentration (LC50) values for ammonia (mg/liter un-ionized NH_3) were 0.5–0.8 for 96 hours, and 0.3–0.6 for 36 days. Nitrite LC50 values (mg/liter NO_2-N) were 0.5–0.6 for 96 hours, and 0.4 for 36 days. Tissues of fish exposed for 29 days to 0.34 mg/liter un-ionized ammonia evidenced degenerative changes in gills, kidneys, and livers. Cutthroat trout fry are comparable to rainbow trout (*Salmo gairdneri*) fry in their susceptibility to acute toxicity from aqueous ammonia and nitrite.

Ammonia can be a serious toxicant to fishes; it enters natural water systems from several sources, including industrial wastes, sewage effluents, agricultural input, and animal feedlots. It is also a metabolic by-product of fishes, and may present a toxicity problem in fish production. A related toxicant is nitrite. As ammonia is converted to nitrate by the process of nitrification, nitrite is formed as an intermediate product. If conversion to nitrate is impeded, significant concentrations of nitrite may accumulate.

Considerable data are available on ammonia toxicity to fishes, some of which have been discussed in a review by Willingham (1976) and in the U.S. Environmental Protection Agency's "Quality Criteria for Water" (1977). Less information is available on the toxicity of nitrite to fishes; this has been summarized by Russo and Thurston (1977). There are no published data on the toxicity of either ammonia or nitrite to cutthroat trout (*Salmo clarki*).

This paper reports the results of eight bioassays, ranging from 11 to 36 days' duration, which were conducted to determine the acute toxicity of ammonia and of nitrite to cutthroat trout fry. Tissues of some of the fish were studied to determine effects of exposure to acutely toxic concentrations of ammonia.

METHODS

Eight bioassays were conducted on cutthroat trout fry (1 g and 3 g); four tested the toxicity of ammonia and four tested nitrite. Eggs, from which the test fish were hatched, were obtained from the Arlee, Montana, State Fish Hatchery, and were reported to be a west slope strain, free from any possible hybridization with rainbow trout (*Salmo gairdneri*). Fish were tested in water from the same ground spring source in which they had been cultured (Table 1), and were acclimated to the bioassay tanks 2–4 days prior to introduction of toxicant. Prior to and during the bioassays, fish were fed a commercial salmon ration three times daily. Fish were weighed collectively at the beginning of the tests, individually as mortalities occurred, and collectively or individually at termination. They were also individually measured for total length as mortalities occurred or at the conclusion of each test.

Reagent grade sodium nitrite or ammonium chloride was used as the toxicant. Toxicant solutions were delivered by proportional diluters having the basic design of Mount and Brungs (1967) at a flow rate of 500 ml every 3–4 min to each of six tanks: five test and one control. Water volume in the tanks was 62 liters. A dilution factor of

TABLE 1.—*Chemical characteristics of the dilution water used in bioassays. All values are mg/liter unless otherwise noted.*

Alkalinity, as $CaCO_3$	176	Al	<1
Hardness, as $CaCO_3$	199	As, µg/liter	1.5
Ca^{2+}	53	Cd	<0.005
Mg^{2+}	36	Cr	<0.005
pH	8.0	Cu	0.008
Cl^-	0.44	Fe	0.03
F^-	0.4	Hg, µg/liter	<0.05
SO_4^{2-}	10.0	K	0.64
PO_4^{3-}	<0.1	Mn	0.003
Turbidity, NTU	1.7	Na	4.0
Specific equivalent conductance, µmho/cm 25 C	328	Ni	<0.005
NH_3-N	0.00	Pb	<0.01
NO_2-N	0.00	Se, µg/liter	0.8
NO_3-N	0.23	Zn	0.01
Total organic carbon	3.3		

0.75 was used for all bioassays, with the exception of one nitrite bioassay on 3-g trout in which the dilution factor was 0.5.

Nitrite-nitrogen (NO_2-N) concentrations were determined according to the method described in Strickland and Parsons (1972). Total ammonia-nitrogen (NH_3-N) was determined using the nesslerization method from APHA (1971). Dissolved oxygen, alkalinity, hardness, and nitrate-nitrogen (NO_3-N) were determined according to analytical procedures described in APHA (1971); for dissolved oxygen the azide modification of the iodometric method was used, with phenylarsine oxide substituted for sodium thiosulfate. Temperature was measured using a (certified) mercury thermometer, and pH was determined with a Beckman Phasar-I digital pH meter. All colorimetric measurements were made using a Varian 635 ultraviolet-visible spectrophotometer.

The averages and ranges of alkalinity over

TABLE 2.—*Acute toxicity of ammonia to cutthroat trout* (Salmo clarki).

Test no.	Mean fish wt. (g)	No. fish per tank	Concentration range tested (mg/liter NH_3-N)	LC50 (95% confidence limits) (mg/liter NH_3-N)			LC50 (95% confidence limits) (mg/liter un-ionized NH_3)		
				4-day	29-day	36-day	4-day	29-day	36-day
178	1.0	20	15.5–55.5	43.6 (40.5–46.9)	30.8 (28.2–33.8)	30.8 (28.2–33.8)	0.80 (0.74–0.86)	0.56 (0.52–0.62)	0.56 (0.52–0.62)
179	1.0	20	12.5–52.5	37.8 (35.7–40.0)	32.2 (29.0–35.7)	32.2 (29.0–35.7)	0.66 (0.62–0.70)	0.56 (0.51–0.62)	0.56 (0.51–0.62)
192	3.3	10	18.7–66.5	36.7 (33.0–40.8)	21.6 (19.4–24.0)		0.62 (0.56–0.69)	0.37 (0.33–0.41)	
193	3.4	10	21.4–65.9	32.4 (29.2–36.9)	21.4		0.52 (0.47–0.59)	0.34	

Test no.	Temp (C) Mean (range)	pH Mean (range)	Dissolved oxygen (mg/liter) Mean (range)
178	13.1 (11.7–14.7)	7.81 (7.74–7.88)	8.6 (7.6–9.2)
179	12.8 (12.0–13.5)	7.80 (7.73–7.93)	8.4 (7.4–9.2)
192	12.4 (11.9–12.7)	7.80 (7.75–7.88)	8.2 (8.0–8.7)
193	12.2 (12.0–12.4)	7.78 (7.72–7.83)	8.3 (7.9–8.6)

TABLE 3.—*Acute toxicity of nitrite to cutthroat trout (*Salmo clarki*).*

Test no.	Mean fish wt. (g)	No. fish per tank	Concentration range tested (mg/liter NO_2-N)	LC50 (95% confidence limits) (mg/liter NO_2-N) 4-day	11-day	36-day	Temp (C) Mean (range)	pH Mean (range)	Dissolved oxygen (mg/liter) Mean (range)
180	1.0	20	0.16–0.50			0.38 (0.33–0.44)	12.4 (11.5–13.0)	7.85 (7.75–7.97)	8.5 (7.8–8.9)
181	1.0	20	0.19–0.59		0.54 (0.47–0.63)	0.37 (0.33–0.42)	12.4 (11.7–13.3)	7.88 (7.78–7.98)	8.6 (7.9–9.0)
195	3.1	10	0.19–2.86	0.56 (0.36–0.79)	0.47 (0.34–0.65)		11.8 (11.7–12.0)	7.88 (7.83–7.96)	8.6 (8.2–8.9)
196	3.1	10	0.29–1.00	0.48 (0.42–0.53)	0.39 (0.33–0.47)		12.1 (11.9–12.3)	7.80 (7.72–7.88)	8.1 (7.3–8.4)

all tests were 178 (175–180) mg/liter $CaCO_3$, and for hardness 200 (195–203) mg/liter $CaCO_3$. For the ammonia tests NO_3-N was 0.08 (0.00–0.17) mg/liter and NO_2-N was 0.00 mg/liter. For the nitrite tests NO_3-N was 0.25 (0.03–0.93) mg/liter and NH_3-N was 0.04 (<0.02–0.06) mg/liter. The ranges of test concentrations of NO_2-N and NH_3-N, and test values of dissolved oxygen, pH, and temperature are listed in Tables 2 and 3.

Mortality observations were made at 4- to 8-h intervals during the first 24 h of each test, 6- to 12-h intervals during the next 3 days of each test, and at no more than 24-h intervals until the tests terminated. The median lethal concentration (LC50) values and their 95% confidence limits were calculated from the experimental data using a computer program developed for the trimmed Spearman-Karber method (Hamilton et al. 1977). Toxicity curves were computer-drawn using data points (LC50 vs. time values) for 55–61 observations for the ammonia tests and 21–32 observations for the nitrite tests. Although the curves do not necessarily go through every LC50 data point, they reflect the best fit for all observations and provide an LC50 estimate for any time included. Un-ionized ammonia (NH_3) concentrations were calculated from NH_3-N concentrations using the formulas of Thurston et al. (1974).

After 29 days' exposure, fish from one ammonia test were preserved for histological examination. Ten fish from the tank containing 21.4 mg/liter NH_3-N (0.34 mg/liter NH_3) and 10 control fish were preserved in Bouin's solution. Gill, heart, intestinal tract, kidney, liver, spleen, and thymus tissues were dissected from each fish, dehydrated, and embedded in paraffin. Sections were cut at 5 μ and stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

Ammonia

Ammonia in aqueous solution assumes an equilibrium between ionized (NH_4^+) and un-ionized (NH_3) chemical species. This equilibrium is principally dependent upon pH and temperature (Thurston et al. 1974; Emerson et al. 1975). The un-ionized ammonia species is toxic to fishes (Chipman 1934; Wuhrmann et al. 1947; Wuhrmann and Woker 1948; Hemens 1966), and the ionized species is considered nontoxic, or is considerably less toxic (Tabata 1962).

Four-day (96-h) LC50 values, with 95% confidence limits, for the four bioassays on ammonia are presented in Table 2. LC50 values are also presented for 29 days, test duration for the 3-g fish, and for 36 days for the 1-g fish. These values are expressed as both total ammonia-nitrogen (NH_3-N) and un-ionized ammonia (NH_3). Average starting weights for fish in the four bioassays were 1.0, 1.0, 3.3, and 3.4 g. The 4-day LC50 values (mg/liter NH_3) for 1-g fish were 0.80 and 0.66; by the conclusion of the tests the LC50 values were both 0.56. The 4-day LC50 values (mg/liter NH_3) for 3-g fish were 0.62 and 0.52, and at the conclusion of the tests the LC50 values were 0.37 and 0.34. The toxicity curves for all four tests (Fig. 1) leveled off after 15–20 days, giving an average asymptotic LC50 of 0.56 mg/liter NH_3

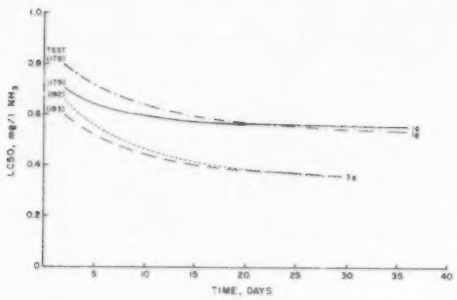

FIGURE 1.—*Toxicity curves (LC50 vs. time) for ammonia bioassays on cutthroat trout fry.*

for the 1-g fish and 0.36 for the 3-g fish. The highest concentrations in each test at and below which no mortalities occurred in 5 days were 15.5 and 12.5 mg/liter NH_3-N (0.28 and 0.22 mg/liter NH_3) for 1-g trout and 24.6 and 21.4 mg/liter NH_3-N (0.42 and 0.34 mg/liter NH_3) for 3-g trout. No mortalities were observed among 1-g trout exposed to 12.5 mg/liter NH_3-N (0.22 mg/liter NH_3) for 36 days.

In all tests there was a negative correlation between ammonia concentration and average weight gain. The Spearman rank correlation coefficient, r_s, (Snedecor and Cochran 1967; Zar 1972) was -0.950 ($P \leq 0.005$) for 1-g fish and -0.886 ($P \leq 0.025$) for 3-g fish.

Tissues from heart, gastrointestinal tract, and thymus of fish exposed to 0.34 mg/liter NH_3 for 29 days were comparable to those of control fish. However, gills and kidneys of exposed fish showed degenerative changes. Gills exhibited hypertrophy of epithelium, some necrosis of epithelial cells, and separation of epithelium due to edema (Figs. 2–4). Kidneys demonstrated mild hydropic degeneration and accumulation of hyaline droplets in renal tubule epithelium (Fig. 5). Reduced vacuolation was observed in livers of the exposed fish.

Published LC50 values for short-term toxicity tests (1–3 days) on salmonids (rainbow trout, *Salmo gairdneri*; Atlantic salmon, *S. salar*) range from 0.2 to 0.5 mg/liter NH_3 (Lloyd and Herbert 1960; Herbert and Shurben 1963, 1965; Danecker 1964; Ball 1967; Lloyd and Orr 1969). Holland et al. (1960) reported that the "critical level" for coho salmon (*Oncorhynchus kisutch*) is 0.12 mg/liter NH_3, and Penaz (1965) states that the limit of damaging effects for brown trout (*S. trutta* var. *fario*) is approximately 0.4 mg/liter NH_3. The ammonia LC50 values for cutthroat trout in the present study are at the upper end of, and slightly higher than, the range reported for other salmonids. This may not be attributable to species differences, but rather to other factors such as fish size. Rainbow trout parr are more tolerant to ammonia than juveniles or adults (Thurston, unpublished data). The ammonia toxicity data reported here for cutthroat trout are similar to data obtained for rainbow trout of the same sizes.

Gill tissue damage is common among fishes exposed to ammonia. The type of lesion varies, depending upon the concentration of ammonia and duration of exposure. Acute exposure results in lesions that are usually degenerative, whereas chronic exposure results primarily in proliferative lesions (Burrows 1964; Flis 1968; Smith and Piper 1975; Smart 1976). The hydropic degeneration demonstrated in the kidneys of exposed fish may be related to an increase in the permeability of fish tissues to water, and increased urine output as described by Lloyd and Orr (1969) for rainbow trout. The hyaline droplets in kidney tubule epithelium suggest reabsorption of excessive amounts of protein from the glomerular filtrate. The reduced vacuolation in the livers of the exposed fish was probably attributable to reduced glycogen and fat storage.

Nitrite

Results of the four bioassays on nitrite are summarized in Table 3. LC50 values are reported for 96 h for the 3-g fish, and are also reported for 11 days which was when the tests terminated. During the tests on 1-g fish insufficient numbers of deaths occurred to determine a valid 96-h LC50. However, an 11-day LC50 for one of the tests was obtainable for comparison with results for 3-g fish. Thirty-six day (test termination) LC50 values are reported for both tests on 1-g fish. LC50 values were in the range of 0.4 to 0.6 mg/liter NO_2-N. The toxicity curves (Fig. 6) show asymptotic LC50 values of approximately 0.4 mg/liter NO_2-N for 1-g fish after

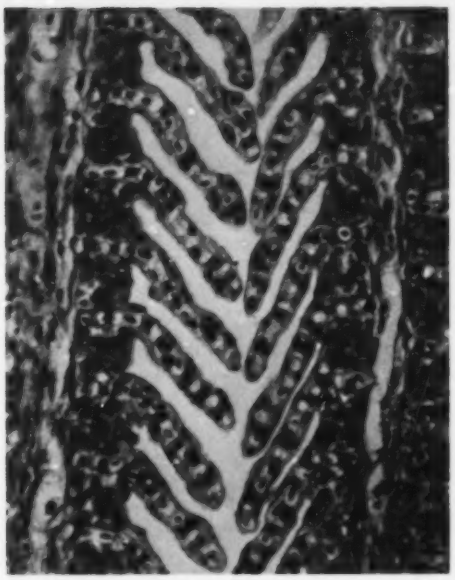

FIGURE 2.—*Normal gill lamellae from control cutthroat trout fry.*

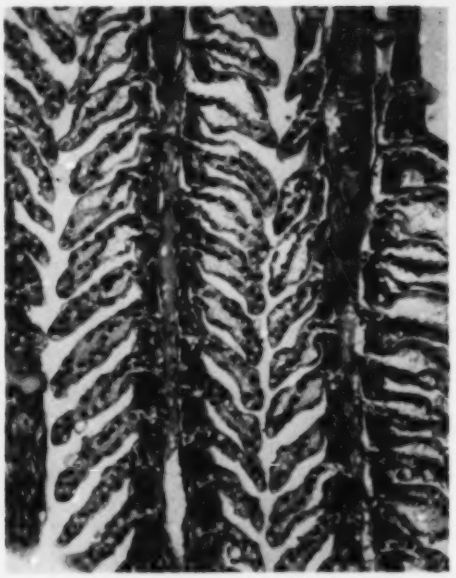

FIGURE 4.—*Cutthroat trout fry exposed to 21.4 mg/liter NH_3-N (0.34 mg/liter NH_3) for 29 days. Gill epithelium is detached from supportive cells and is edematous.*

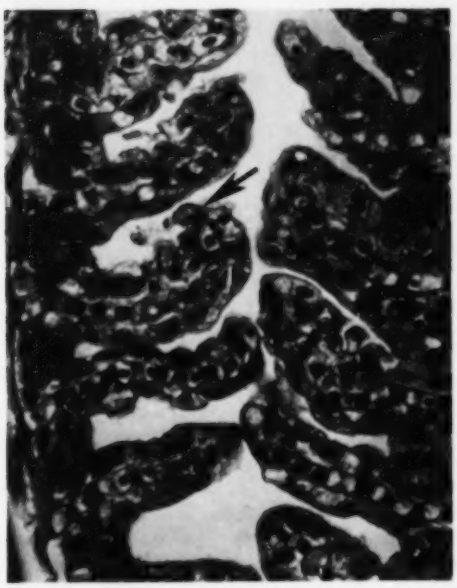

FIGURE 3.—*Cutthroat trout fry exposed to 21.4 mg/liter NH_3-N (0.34 mg/liter NH_3) for 29 days. Lamellae show hypertrophy and scattered necrosis of gill epithelium (arrow).*

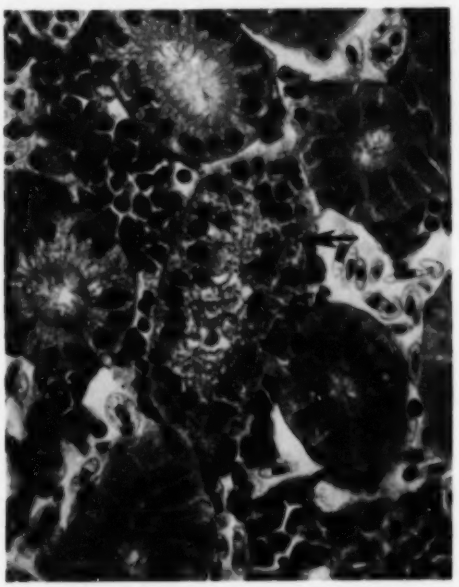

FIGURE 5.—*Cutthroat trout fry exposed to 21.4 mg/liter NH_3-N (0.34 mg/liter NH_3) for 29 days. Renal tubule epithelium showing hyaline droplet degeneration (arrow).*

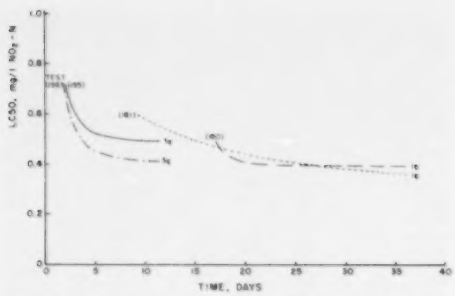

FIGURE 6.—*Toxicity curves (LC50 vs. time) for nitrite bioassays on cutthroat trout fry.*

about 20–25 days and 0.4–0.5 for 3-g fish after 8–10 days. The maximum concentration at which fish survived with no mortalities for the duration of the longer (36-day) tests was 0.20 and 0.25 mg/liter NO_2-N.

There was no significant difference between the two fish sizes tested in their susceptibility to nitrite. Among fish that survived for the entire test periods there was also no significant difference in average weight or length between control fish and test fish at any nitrite concentration. Most of the test fish that succumbed changed from their normal light gray color to black prior to death. Surviving fish evidenced a correlation between degree of darkened coloration and nitrite concentration.

The 96-h LC50 concentrations reported for this study are slightly higher and comparable to that (0.4 mg/liter NO_2-N) found to be toxic to rainbow trout of a similar size (2 g) tested under similar conditions (Russo et al. 1974). Brown and McLeay (1975) reported a 96-h LC50 of 0.23 mg/liter NO_2-N for 9-g rainbow trout, and Russo et al. (1974) and Russo and Thurston (1977) reported 96-h LC50 values of 0.2–0.3 mg/liter in eight tests on rainbow trout ranging in size from 12 to 235 g. All of these reported LC50 results on salmonids are in close agreement, and for identical time periods; values fall within the range of 0.2–0.4 mg/liter NO_2-N for *S. gairdneri*, and 0.4–0.6 for *S. clarki*.

For a shorter time period of 24 h, Smith and Williams (1974) conducted two tests on rainbow trout; with 4.5-g fish, 50% died at 1.6 mg/liter NO_2-N, and with 100-g fish, 55% died at 0.55 mg/liter. Our 24-h LC50 values for the 3-g cutthroat trout in the present study were 1.16 and 0.95 mg/liter NO_2-N. We also see little difference between these results for cutthroats and those for rainbow for a 24-h time period.

Nitrite toxicity data are available in the literature for one other salmonid, the chinook salmon (*Oncorhynchus tshawytscha*). Smith and Williams (1974) reported 40% mortality in 24 h for 32-g fish exposed to 0.50 mg/liter NO_2-N, and Westin (1974) reported a 96-h LC50 value of 2.9 mg/liter NO_2^- (0.88 mg/liter NO_2-N) for a test on a group of chinooks that ranged in size from 1.5 to 10.6 g. Westin's study, in different dilution water and over a longer time period, showed a somewhat greater tolerance for nitrite by chinook salmon than found by Smith and Williams. Crawford and Allen (1977) reported a 48-h LC50 of 19 mg/liter NO_2^- (5.8 mg/liter NO_2-N) for chinooks averaging 10.2 g in a freshwater static bioassay.

In addition to differences in susceptibility to nitrite toxicity depending upon stage of development (fry vs. fingerlings and larger fish), test water differences must also be considered when comparisons are made. Crawford and Allen (1977) considered the possible effect of calcium on nitrite toxicity, and the inhibitory effect of chloride has recently been demonstrated by Russo and Thurston (1977) and Perrone and Meade (1977). Chloride was not measured during the present study. Based on chloride measurements on dilution water from the same source, prior to and since this study, we have no reason to believe that chloride exceeded 0.5 mg/liter at any time.

The differences between the results of Westin (1974) and Smith and Williams (1974), both working on the same species, might be reconciled by considering the chlorinity factor. Smith and Williams used test water from the same groundwater source described in Table 1, which had chloride levels below 0.5 mg/liter. Westin used a reconstituted laboratory test water (Lennon and Walker 1964) that included 1.4 mg/liter chloride. From the data obtained by Russo and Thurston (1977) on the inhibiting effect of chloride on nitrite toxicity to rainbow trout, Westin's LC50 value can be reduced

SMART, G. 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). J. Fish Biol. 8:471–475.

SMITH, C. E., AND R. G. PIPER. 1975. Lesions associated with chronic exposure to ammonia. Pages 497–514 *in* W. E. Ribelin and G. Migaki, eds. The pathology of fishes. University of Wisconsin Press, Madison.

———, AND W. G. WILLIAMS. 1974. Experimental nitrite toxicity in rainbow trout and chinook salmon. Trans. Am. Fish. Soc. 103:389–390.

SNEDECOR, G. W., AND W. G. COCHRAN. 1967. Statistical methods, 6th ed. Iowa State Univ. Press, Ames. 593 pp.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. Fish. Res. Board Can. Bull. 167:77–80.

TABATA, K. 1962. Toxicity of ammonia to aquatic animals with reference to the effect of pH and carbon dioxide. Bull. Tokai Reg. Fish. Res. Lab. 34:67–74. [English translation.]

THURSTON, R. V., R. C. RUSSO, AND K. EMERSON. 1974. Aqueous ammonia equilibrium calculations. Tech. Rep. 74-1, Fisheries Bioassay Laboratory, Montana State University, Bozeman. 18 pp.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1977. Quality criteria for water. Office of Water Planning and Standards, U.S. EPA, Washington, D.C. 256 pp.

WESTIN, D. T. 1974. Nitrate and nitrite toxicity to salmonoid fishes. Prog. Fish-Cult. 36(2):86–89.

WILLINGHAM, W. T. 1976. Ammonia toxicity. EPA-908/3-76-001, U.S. Environ. Prot. Agency Control Technology Branch, Water Division, Region VIII, Denver. 103 pp.

WUHRMANN, K., AND H. WOKER. 1948. Beiträge zur Toxikologie der Fische. II. Experimentelle Untersuchungen über die Ammoniak- und Blausäurevergiftung. (Contributions to the toxicology of fish. II. Experimental investigations on the toxicity of ammonia and hydrocyanic acid.) Schweiz. Z. Hydrol. 11:210–244. [In English translation.]

———, F. ZEHENDER, AND H. WOKER. 1947. Über die fischereibiologische Bedeutung des Ammonium- und Ammoniakgehaltes fliessender Gewässer. (Biological significance for fisheries of ammonium ion and ammonia content of flowing bodies of water.) Vierteljahrsschr. Naturforsch. Ges. Zuerich 92:198–204. [In English translation.]

ZAR, J. H. 1972. Significance testing of the Spearman rank correlation coefficient. J. Am. Stat. Assoc. 67(339):578–580.

by a factor of about 2, which would give an adjusted 96-h LC50 value of approximately 0.4 mg/liter NO_2-N, slightly lower than that of Smith and Williams. The results of the tests by Crawford and Allen are an order of magnitude greater than those reported by other authors.

Summary

Cutthroat trout fry are comparable to rainbow trout fry in their susceptibility to acute toxicity from aqueous ammonia and nitrite. Results of tests in our laboratory also compare favorably to results of most other researchers who have worked with other salmonids under freshwater test conditions. In fresh water, the reported median lethal concentrations for acute toxicity tests on salmonids generally fall within the range 0.2–0.8 mg/liter NH_3, as compared to values between 0.4 and 4 mg/liter NH_3 for other fishes. Also under nonsaline conditions, the median lethal concentrations for nitrite tests on salmonids fall within the range 0.2–0.6 mg/liter NO_2-N, as compared to values from 2 to >67 mg/liter NO_2-N for other fishes (Russo and Thurston 1977).

ACKNOWLEDGMENTS

This work was funded by the U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Research Grants No. R800861 and R803950. Robert J. Luedtke and Catheryn M. Bennett provided valuable assistance in the biological tests and chemical analyses.

REFERENCES

APHA (AMERICAN PUBLIC HEALTH ASSOCIATION), AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Washington, D.C. 874 pp.

BALL, I. R. 1967. The relative susceptibilities of some species of freshwater fish to poisons. I. Ammonia. Water Res. 1:767–775.

BROWN, D. A., AND D. J. MCLEAY. 1975. Effect of nitrite on methemoglobin and total hemoglobin of juvenile rainbow trout. Prog. Fish-Cult. 37(1):36–38.

BURROWS, R. E. 1964. Effects of accumulated excretory products on hatchery-reared salmonids. Bur. Sport Fish. Wildl. (U.S.) Res. Rep. 66. 12 pp.

CHIPMAN, W. A., JR. 1934. The role of pH in determining the toxicity of ammonia compounds. Ph.D. Thesis. Univ. of Missouri, Columbia. 153 pp.

CRAWFORD, R. E., AND G. H. ALLEN. 1977. Seawater inhibition of nitrite toxicity to chinook salmon. Trans. Am. Fish. Soc. 106(1):105–109.

DANECKER, E. 1964. Die Jauchevergiftung von Fischen—eine Ammoniakvergiftung. (The jauche poisoning of fish—an ammonia poisoning.) Ostereichs Fischerei 3/4:55–68. [In English translation.]

EMERSON, K., R. C. RUSSO, R. E. LUND, AND R. V. THURSTON. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32(12):2379–2383.

FLIS, J. 1968. Anatomicohistopathological changes induced in carp (*Cyprinus carpio* L.) by ammonia water. Part I. Effects of toxic concentrations. Acta Hydrobiol. 10:205–224. Part II. Effects of subtoxic concentrations. Ibid. 10:225–238.

HAMILTON, M. A., R. C. RUSSO, AND R. V. THURSTON. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11(7):714–719.

HEMENS, J. 1966. The toxicity of ammonia solutions to the mosquito fish (*Gambusia affinis* Baird & Girard). Proc. Inst. Sewage Purif. 65:265–271.

HERBERT, D. W. M., AND D. S. SHURBEN. 1963. A preliminary study of the effect of physical activity on the resistance of rainbow trout (*Salmo gairdnerii* Richardson) to two poisons. Ann. Appl. Biol. 52:321–326.

———, AND ———. 1965. The susceptibility of salmonid fish to poisons under estuarine conditions. II. Ammonium chloride. Int. J. Air Water Pollut. 9:89–91.

HOLLAND, G. A., J. E. LASATER, E. D. NEUMANN, AND W. E. ELDRIDGE. 1960. Toxic effects of organic and inorganic pollutants on young salmon and trout. Wash. Dep. Fish. Res. Bull. 5:183–187.

LENNON, R. E., AND C. R. WALKER. 1964. Laboratories and methods for screening fish-control chemicals. Invest. Fish Control No. 1. 15 pp.

LLOYD, R., AND D. W. M. HERBERT. 1960. The influence of carbon dioxide on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdnerii* Richardson). Ann. Appl. Biol. 48:399–404.

———, AND L. D. ORR. 1969. The diuretic response by rainbow trout to sub-lethal concentrations of ammonia. Water Res. 3:335–344.

MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1:21–29.

PENAZ, M. 1965. Vliv amoniaku na jikry a plůdek pstruha obecného, *Salmo trutta* m. *fario*. (The influence of ammonia on the eggs and young of the common trout, *Salmo trutta* var. *fario*.) Zool. Listy 14:47–54. [In English translation.]

PERRONE, S. J., AND T. L. MEADE. 1977. Protective effect of chloride on nitrite toxicity to coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 34(4):486–492.

RUSSO, R. C., C. E. SMITH, AND R. V. THURSTON. 1974. Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31(10):1653–1655.

———, AND R. V. THURSTON. 1977. The acute toxicity of nitrite to fishes. Pages 118–131 *in* R. A. Tubb, ed. Recent advances in fish toxicology. U.S. Environ. Prot. Agency Ecol. Res. Ser. EPA-600/3-77-085, Corvallis, Oregon.